Geographical variation in male genitalia in *Brachyrhaphis episcopi* (Poeciliidae): is it sexually or naturally selected?

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Male poeciliid fishes inseminate females using an intromittent organ called the gonopodium. Here we report on natural variation in gonopodium size both within and between 12 populations of the freshwater fish *Brachyrhaphis episcopi* (Poeciliidae) in Panama. We show that males from sites with more predatory fish species have, on average, a relatively longer gonopodium than males inhabiting sites with fewer predatory fish. Gonopodium length was not correlated with the site-specific adult sex ratio and the average sex ratio was more strongly female biased at sites with more predatory fish. The gonopodium exhibited lower phenotypic variance than the average for sexually selected traits and it generally showed negative allometry. Our results are similar to those reported for the guppy *Poecilia reticulata*. Two alternative hypotheses for these findings are discussed. First, that population differences are sexually selected. Second, that they are an incidental consequence of environmental differences between sites. Specifically, that higher water flow rates select for enlarged fin size and stockier bodies in downstream sites where predatory fish are more common.

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The morphology of intromittent genitalia varies intraspecifically for many animals (e.g. Arnqvist and Thornhill 1998), including a poeciliid fish (Kelly et al. 2000). Three main hypotheses have been offered to explain the evolutionary processes responsible for genitalic evolution (see Arnqvist 1997, Eberhard 1985 for reviews). First, the lock-and-key hypothesis posits that pre-mating mechanical isolation selects for male genitalia that are species-specific and fit appropriately in female genitalia. Despite its historical popularity, recent comparative studies have failed to support the predictions of this hypothesis (reviewed in Eberhard 1985). Second, Mayr (1963) proposed the 'pleiotropy hypothesis' in which he argued that high inter-specific variation in genital morphology is an indirect result of evolution of genetically correlated characters, and that genitalia do not figure prominently in pre-mating mechanical isolation. This hypothesis carries the implicit assumption that variation in genitalic morphology is close to selectively neutral. It has not yet received strong empirical support and is difficult to test (Eberhard 1985). Third, Eberhard (1985, 1993, 1996) suggested that copulatory and post-mating sexual selection is responsible for rapid and divergent evolution of genital traits. Morphological variation of genital traits among males that causes variation in paternity success may derive from three different, but not mutually exclusive, sexual selection mechanisms: sperm competition (Birkhead and Møller 1998); cryptic female choice (Eberhard 1985, 1996); and conflict between the sexes over the control of fertilisation (Arnqvist and Rowe 1995, Alexander et al. 1997). Several lines of evidence directly implicate sexual selection in the evolution of genitalia (e.g. Arnqvist 1998, Arnqvist et al. 2000; review: Eberhard 1996).

Although long assumed that directional sexual selection exhausts additive genetic variation ('the lek paradox') (Taylor and Williams 1982), sexually selected display traits generally show high within-species phenotypic variation (e.g. Cuervo and Møller 1999), and heritability (Pomiankowski and Møller 1995). This has been attributed to their expression being condition-dependent (Rowe and Houle 1996, Wilkinson and Taper 1999). Sexually selected display traits also tend to exhibit positive allometry (Petrie 1988, 1992, Møller 1991, Green 1992) that further increases estimates of total phenotypic variation uncorrected for body size. Eberhard et al. (1998) found, however, that intromittent organs in insects and spiders have lower allometric slopes than other body parts and show slightly less phenotypic variation. They argue that a female's own size influences her perception of stimulatory traits that contact her reproductive tract in precise ways selecting for reduced variation in genital size. It is unclear if this difference between sexually selected display versus genitalic traits is widespread. For example, there may be taxa where females assess male quality through genital size (Arnqvist 1997), or genital size increases insemination success during male-male competition (Eberhard et al. 1998) or male-female conflict (Alexander et al. 1997). Both mechanisms could lead to condition-dependence and positive allometry of genitalia.

To date, the few available studies examining the evolution of male genital traits by sexual selection have focused on insects. Little is known about within-species variation in vertebrate genitalia (Kelly et al. 2000). Like most poeciliid fish, *Brachyrhaphis episcopi* has internal fertilisation and males inseminate females using a modified, elongated anal fin called a gonopodium (Rosen and Gordon 1953). We first calculated the allometry of gonopodial length in *B. episcopi*. We then tested whether population variation in gonopodium length is associated with predator community type and/ or the adult sex ratio. We examined these two factors because there is sexual conflict over insemination in *B. episcopi*. First, although courtship has been reported from captive fish (D. Fromm pers comm), males mainly attempt to coerce females into mating without engaging in courtship ('copulatory thrusting' or 'sneak mating') (Bisazza 1993). Similar to other poeciliids (e.g. Trinidadian guppy, *Poecilia reticulata*, Kelly et al. 1999; mosquitofish, *Gambusia holbrooki*, Bisazza et al. 2001), female *B. episcopi* may only be in partial control of the number of sneak matings they receive. As the sex ratio becomes more male-biased there should be greater competition among male *B. episcopi* to gain access to females, and sneak mating should increase as seen in guppies (e.g. Evans and Magurran 1999, Jirotkul 1999). Male harassment appears to carry costs for poeciliid females (e.g. Magurran and Seghers 1994a), so female *B. episcopi* should be under stronger selection to avoid males. This, in turn, should increase selection on males

to achieve successful insemination. Second, predation risk often influences sexual behaviour in poeciliids (e.g. *P. reticulata*, Godin 1995, Godin and Briggs 1996). In guppies, for example, males from high predation-risk populations spend less time in anti-predator behaviour and more time trying to sneak mate (Endler 1987, Magurran and Seghers 1994b). Given the association between predation risk, rate of sneak mating and gonopodium length in the guppy, Kelly et al. (2000) suggested that increased predation risk within populations may select for longer gonopodia because males may more readily sneak inseminate females from a distance with longer genitalia. Of course, correlation does not elucidate causation and, at present, the selective forces driving gonopodium elongation in the guppy are unknown. It is also unclear whether population differences are due to genetic differences among populations, or adaptive (or non-adaptive) phenotypic plasticity. However, if the same association between predator-community type and gonopodium length occurs in *B. episcopi* this at least strengthens the case that the association seen in the guppy is more general. Alternatively, a shorter gonopodium may be adaptive for male *B. episcopi* originating from high-predation sites because a shorter structure may be more manoeuvrable and/or more efficient at transferring sperm.

Methods

Brachyrhaphis episcopi is endemic to central Panama (Turner 1938, Loften 1965). At some sites the only predatory fish species is the small killifish *Rivulus brunneus* (Cyprinodontidae), a primarily insectivorous, small-gaped fish that is incapable of eating adult *B. episcopi* (Angermeier and Karr 1983). Other sites contained *R. brunneus* and several, larger predatory fish species (e.g. *Aequidens coeruleopunctatus*, *Piabucina panamensis*, *Brycon* spp. and *Hoplias microlepsis*). Stomach content analysis shows these species all include fish in their diet (Angermeier and Karr 1983, Kramer and Bryant 1995). We classified five sites as *Rivulus* localities because the only predatory fish seen was *R. brunneus*. Seven sites with larger predatory fish were classified as *Characin* localities as Characiformes fish (Lebiasinidae or Characidae) were present. There are significant phenotypic differences in life-history traits between *Rivulus* and *Characin* sites: adults mature sooner and, on average, produce larger broods of smaller offspring at *Characin* sites (Jennions and Telford 2002). These life-history differences are similar to those between high and low predation risk sites in two other poeciliids: the guppy in Trinidad (e.g. Reznick and Endler 1982, Reznick 1989) and *B. rhabdophora* in Costa Rica (Johnson and Belk 2001).

Field and laboratory procedures

We collected 23–62 male *B. episcopi* from each of five *Rivulus* and seven *Characin* sites at the end of the dry season in April–May 1998. Ten sites were on streams under closed-canopy rainforest in the Parque National Soberania that drain into Gatun Lake, the Panama Canal or intermediate lakes. All fish were caught using a hand-held dipnet and immediately anaesthetised with MS-222 (3-aminobenzoic acid ethyl ester) and preserved in 5% formalin. We classified males as sexually mature if they possessed a fully developed gonopodium (Rosen and Gordon 1953). We collected from two sites on each of Quebrada Juan Grande (9°8′36″ N, 79°43′0″ W; 9°8′31″ N, 79°42′43″ W), Rio Frijolito (9°9′35″ N, 79°43′29″ W; 9°9′59″ N, 79°43′29″ W), and R. Mendoza (9°10′3″ N, 79°45′10″ W; 9°11′19″ N, 79°44′29″ W), three sites on R. Macho (9°10′58″ N, 79°45′36″ W; 9°10′56″ N, 79°45′51″ W; 9°10′56″ N, 79°46′0″ W) and a single site on Q. Sardinilla (9°5′26″ N, 79°39′44″ W). For detailed descriptions of the downstream sections of these streams see Angermeier and Karr (1983). All sites on the same stream were separated by waterfalls, which are major physical barriers to upstream fish movement (Loften 1965, pers. obs.). In addition, *B. episcopi* is an upstream species that is replaced downstream by the congener *B. cascajensis* (Loften 1965, Mojica et al. 1997, pers. obs.). We therefore consider different streams to represent different populations and, as with similar studies (e.g. Johnson and Belk 2001), treat sites on the same stream separated by waterfalls as statistically independent sites. We also collected from the R. Anton and R. Mato Ahogado (8°37′50″ N, 80°8′11″ W and 8°38′5″ N, 80°5′35″ W, respectively). These two streams are forested in their uppermost reaches, but only partially shaded at our nearby collection sites. Five *Characin* sites were below waterfalls; one was above (R. Frijolito 1) and one was at the stream's headwaters (Q. Sardinilla). Four *Rivulus* sites were above waterfalls (Q. Juan Grande 1, R. Mendoza 1, R. Macho 1, R. Anton) and the fifth near the stream's headwaters (R. Mato Ahogado). With the exception of R. Frijolito 1, where the only predators were *P. panamensis* and *R. brunneus*, all *Characin* sites contained several piscivorous fish species.

To avoid inter-observer differences, all measurements of the sampled male fish were taken by only one of us (MDJ) and without knowledge of the population of origin. Using dial callipers, the standard body length of each male was measured to the nearest 0.1 mm under a large, desk-mounted magnifying glass. The gonopodium was measured, from the last horizontal segment at its base to the pointed distal tip, at 120 × to the nearest 0.084 mm using a WILD M5A dissecting microscope and ocular micrometer. All males were repositioned (rotated 180°) between successive measurements.

Following Yezerinac et al. (1992), two measures of standard length and gonopodium length were taken, and the mean value for each trait was used in further analysis. We used a model II ANOVA to estimate the repeatability of our measurements (Lessells and Boag 1987, Yezerinac et al. 1992, Sokal and Rohlf 1995). These calculations were performed using all males from one randomly selected site (R. Macho 1, $n = 50$). All measurements were highly repeatable ($r = 0.997$ and 0.995 for gonopodium length and body length, respectively) and measurement errors were very small (% ME = 0.33 and 0.48 for gonopodium length and body length, respectively). Measurement error is defined as that portion of the total sample variance comprised of within-individual variation (Yezerinac et al. 1992). There is considerable among-male variation in body length (range: 14.3–30.6 mm). A year later, we also measured caudal peduncle width, eye diameter and wet weight (± 1 mg), and re-measured standard length. Our measure of mean standard length per population was almost identical across the two measuring periods ($r = 0.996$, $P < 0.001$, $n = 12$).

Statistical analysis

Measurements of both body length and gonopodium length were log$_{10}$ transformed to meet the assumption of normality for ANCOVA (Sokal and Rohlf 1995). The coefficient of variation for both body length and gonopodium length was calculated separately using untransformed data.

Gonopodium length and standard body length were significantly positively correlated at all 12 sites (Table 1). Male body length differed significantly between sites of different predator-community types ($F_{1,452} = 85.1$, $P < 0.0001$) (Jennions and Telford 2002), and among sites within predator-community type ($F_{10,452} = 20.8$, $P < 0.0001$). Therefore, we included standard length as a covariate in our analyses. We performed nested ANCOVA using the GLM procedure of SYSTAT 5.0.

Predator-community type (*Rivulus* or *Characin*) was the main effect, with site nested within predator-community type and treated as a fixed effect because the predator-community type was known a priori and, thus, each site was chosen because of its predator assemblage (see Reznick 1989, Bennington and Thayne 1994). We first performed separate Model I regression for each site and removed any outliers (studentised residuals > 3.0 s.d.: two from R. Frijolito 1, one from Q. Sardinilla and three from R. Anton).

To reduce some problems of interpretation arising from among-site differences in the relationship between body and gonopodium length (slope heterogeneity), and to control for possible variation across streams, we compared *Rivulus* and *Characin* sites within three streams: R. Macho, R. Mendoza and Q. Juan Grande.

For R. Macho, the comparison was between the two most distant sites. We ran separate ANCOVA models for each stream. In each case there was no significant slope heterogeneity and the interaction term was removed from the final model (Sokal and Rohlf 1995). Finally, we compared the two *Characin* localities on R. Frijolito. Rio Frijolito 1 is above, and R. Frijolito 2 is below, a waterfall. If differences between *Rivulus* and *Characin* localities are due to above/below waterfall differences (irrespective of predator regime) then differences between R. Frijolito 1 and 2 should mirror those between *Rivulus* and *Characin* localities. Following Rice (1989), we used the sequential Bonferroni correction to maintain a table-wide type-I error rate of 0.05 because four tests of the main effect of predator-type locality were carried out (p critical $= 0.0125$). Unless otherwise stated, all tests are two-tailed and data are presented as mean $\pm$ s.d. Differences are expressed as a percentage of the smaller value. Adjusted least-squares means (LSM) are presented for GLM models.

Results

Gonopodium length and predator-community type

There was a significant difference among sites in gonopodium length ($F_{10,440} = 83.2$, $P < 0.0001$). Length also varied significantly among sites within predator-community type (*Rivulus*: $F_{4,194} = 50.2$, $P < 0.0001$ and *Characin*: $F_{6,246} = 105$, $P < 0.0001$). The male gonopodium (adjusted for body length) was significantly longer on average ($F_{1,440} = 12.4$, $P = 0.0005$) for males originating from *Characin* sites (adjusted mean =

6.25 mm, $n = 260$) compared with males from *Rivulus* sites (adjusted mean $= 6.16$ mm, $n = 204$; Table 1, Fig. 1). This represents a relative difference of 1.4% in mean gonopodium length between the two predator-community types (Fig. 1).

The full model, however, indicated a weak but significant interaction between site and body length ($F_{11,440} = 1.98$, $P = 0.028$) which precluded a straightforward interpretation of the results. The relationship between body length and gonopodium length did not differ among the seven *Characin* sites ($F_{6,246} = 0.65$, $P = 0.69$) but did differ among the five *Rivulus* sites ($F_{4,194} = 4.06$, $P = 0.004$). We excluded the site with the highest (Anton) and the lowest (Sardinilla) slope coefficients from the analysis to reduce slope heterogeneity ($F_{9,394} = 1.39$, $P = 0.19$). The former is a *Rivulus* and the latter a *Characin* site. Gonopodium length was still significantly longer by 3.3% at *Characin* sites ($F_{1,403} = 99.0$, $P < 0.0001$; LSM: 6.34 vs 6.14 mm, $n = 236, 178$). Quebrada Sardinilla is also an outlier among *Characin* sites (> 3.0 s.d. below the mean). Its exclusion alone resulted in a difference between predator-community types of 3.7%, ($F_{1,418} = 75.5$, $P < 0.0001$; LSM: 6.39 vs 6.16 mm, $n = 236$ and 204, respectively; Fig. 1).

Predator-community type and sex ratios

The mean ratio of adult females to males in the wet season was 2.36 ± 0.79 and in the dry season 2.02 ± 0.92. The adult sex ratio did not differ between seasons (Wilcoxon test, $Z = 0.94$, $P = 0.35$) and both were significantly greater than 1:1 ($t_{11} = 5.98$, $P < 0.001$ and

Table 1. Model I regression of $\log_{10}$ gonopodium length on $\log_{10}$ standard body length for each of twelve *Brachyrhaphis episcopi* sites (site identity in parentheses). RMA = Reduced major axis regression. The t-tests are for the null hypothesis that slope $= 1$. * $P < 0.05$ following sequential Bonferroni correction.

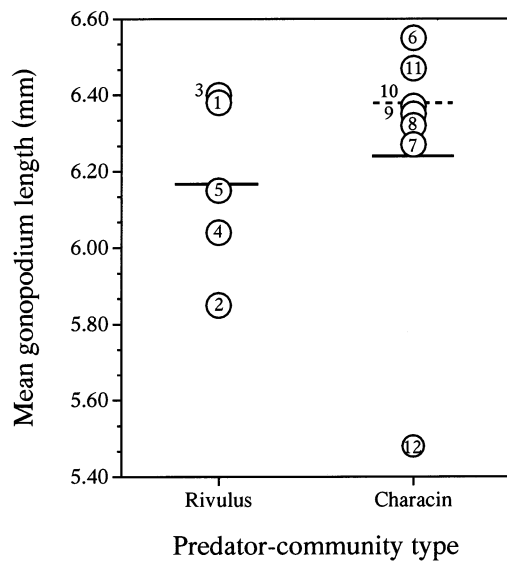| Site	n	Model I Slope (SE)	Model I Intercept (SE)	Model I t-value (P)	RMA slope	RMA t-value (P)	Least-squares mean (mm)
Rivulus sites							
Juan Grande 1 (1)	39	0.903 (0.032)	-0.384 (0.042)	3.03 (0.004) *	0.923	2.03 (0.05)	6.38
Mendoza 1 (2)	51	0.958 (0.053)	-0.495 (0.068)	0.790 (0.43)	1.028	-0.47 (0.64)	5.85
Macho 1 (3)	62	0.810 (0.025)	-0.260 (0.035)	7.64 (<0.0001) *	0.833	6.92 (<0.0001) *	6.40
Rio Anton (4)	26	1.026 (0.054)	-0.570 (0.075)	-0.477 (0.64)	1.061	-1.11 (0.28)	6.04
Mato Ahogado (5)	26	0.979 (0.087)	-0.501 (0.118)	0.24 (0.81)	1.065	-0.75 (0.46)	6.15
Mean ($n = 5$)		0.935	-0.442		0.982		6.16
Characin sites							
Juan Grande 2 (6)	42	0.878 (0.035)	-0.341 (0.045)	3.49 (0.0012) *	0.905	2.86 (0.007)	6.55
Frijolito 1 (7)	43	0.905 (0.039)	-0.395 (0.045)	2.69 (0.01) *	0.932	1.74 (0.089)	6.27
Frijolito 2 (8)	36	0.835 (0.042)	-0.298 (0.054)	3.92 (0.0004) *	0.870	2.98 (0.005) *	6.32
Mendoza 2 (9)	33	0.846 (0.044)	-0.311 (0.058)	3.50 (0.0014) *	0.880	2.75 (0.01)	6.35
Macho 2 (10)	36	0.882 (0.051)	-0.358 (0.068)	2.31 (0.027)	0.930	1.61 (0.12)	6.37
Macho 3 (11)	46	0.888 (0.030)	-0.358 (0.040)	3.73 (0.005) *	0.909	3.03 (0.004) *	6.47
Sardinilla (12)	24	0.805 (0.044)	-0.321 (0.058)	4.47 (<0.0001) *	0.830	3.86 (<0.0001) *	5.48
Mean ($n = 7$)		0.863	-0.340		0.894		6.25

Fig. 1. Mean gonopodium length of males (adjusted for individual body standard length) originating from each of twelve *Brachyrhaphis episcopi* sites which were categorised as *Rivulus* or *Characin* predator-community types. Numbers inside the data points denote individual site identities (see Table 1). Mean adjusted gonopodium length was longer for males originating from *Characin* sites than males from *Rivulus* sites when Q. Sardinilla was both excluded (dashed line; nested ANCOVA, $P < 0.0001$, $n = 11$) and when it was not (solid line; $P = 0.0005$, $n = 12$).

$t_{11} = 3.85$, $P = 0.003$, respectively). There was, however, no correlation between the adult sex ratio at a site in the wet and dry season ($r = -0.125$, $P = 0.70$, $n = 12$). Using the mean sex ratio per site, there were significantly more females per male at the *Characin* than *Rivulus* sites (Mann-Whitney test, $U = 5$, $P = 0.04$; 2.47 ± 0.54 vs 1.80 ± 0.33). There was, however, no correlation between relative gonopodium length and mean adult sex ratio ($r = 0.197$, $P = 0.54$, $n = 12$).

Coefficient of variation

The mean CV for gonopodium length was 10.78% (7.67–14.10), for caudal peduncle width 11.66% (8.88–15.02), for eye width 10.29% (6.18–13.30%) and for body mass 36.13% (22.68–48.39%). Neither variation in gonopodium nor body length differed significantly between *Characin* and *Rivulus* sites (Mann-Whitney tests, $U = 11$, $P = 0.29$, $U = 9$, $P = 0.17$). Although very similar, the mean CV for gonopodium length was significantly smaller than that for caudal peduncle width (Wilcoxon test, $Z = 2.59$, $P = 0.01$). The CV for gonopodium length and eye width did not differ (Wilcoxon test, $Z = 1.18$, $P = 0.24$). However, a comparison of adjusted r^2 values (which corrects for the contribution of slope allometry to the CV) for the regressions for eye width and gonopodium length

showed that body length explained far less of the variation in eye width (Wilcoxon test, $Z = 3.06$, $P = 0.002$; $76.1 \pm 10.8\%$ vs $92.0 \pm 3.6\%$). *Brachyrhaphis episcopi* had a significantly larger mean CV for gonopodium length than *P. reticulata* [two-sample *t*-test, $t = 4.95$, df = 18, $P = 0.0001$; data for the gonopodium length CV for each of eight *P. reticulata* populations originate from the Kelly et al. (2000) study].

Allometric relationships

The slopes of the Model I regression of gonopodium length on standard length were significantly less than unity at nine sites (eight following sequential Bonferroni corrections). Overall, the mean slope was 0.893 ± 0.068 which is significantly less than unity ($t_{11} = 5.50$, $P = 0.0002$). These trends were mirrored when we used reduced major axis regression where the mean slope of 0.931 ± 0.081 was also significantly less than unity ($t_{11} = 2.98$, $P = 0.012$). The mean allometric slope was also less than unity for eye width ($t_{11} = 13.24$, $P < 0.0001$; 0.76 ± 0.06), but not for caudal peduncle width ($t_{11} = 1.49$, $P = 0.164$; 0.97 ± 0.08). The mean slope for eye width was less than that for gonopodium length (Wilcoxon test, $Z = 2.98$, $P = 0.003$).

To compare body shape between *Rivulus* and *Characin* sites we tested for differences in caudal peduncle width or body mass corrected for body length. Mean caudal width was significantly greater by 1% at *Characin* sites ($F_{1,451} = 5.66$, $P = 0.02$; 6.82 vs 6.76 mm), but there was no difference in body mass ($F_{1,449} = 3.57$, $P = 0.06$; *Rivulus* 198.0 vs *Characin* 197.0 mg).

Within-stream comparisons

Mean adjusted gonopodium length was significantly longer at *Characin* than *Rivulus* sites on both Q. Juan Grande ($F_{1,78} = 13.6$, $P < 0.0001$; 6.37 vs 6.21 mm) and R. Mendoza ($F_{1,81} = 121$, $P < 0.0001$; 6.02 vs 5.50 mm). There was moderate slope heterogeneity between sites on R. Macho ($F_{1,104} = 4.05$, $P = 0.047$). However, across 95% of the actual range in male size, gonopodium length was greater at the *Characin* site ($F_{1,104} = 12.0$, $P = 0.0008$, 6.93 vs 6.81 mm). Finally, mean gonopodium length was significantly longer at the downstream than the upstream *Characin* sites on R. Frijolito ($F_{1,76} = 8.74$, $P = 0.004$; 5.70 vs 5.60 mm).

Discussion

Gonopodium length in *B. episcopi* varied significantly among sites and there was an association between predator community and relative gonopodium length.

Males from sites with large fish predators have, on average, significantly longer gonopodia than males from sites with fewer predators. The relative difference in gonopodium length between the two predator-community types was 1.4% (all sites), 3.3% (removing two sites to decrease slope heterogeneity) or 3.7% (excluding an outlier site). These results cannot be attributed to measurement error. There was no correlation between male gonopodium length and the site-specific adult sex ratio, but the mean sex ratio was more female-biased at *Characin* sites. A more female-biased sex ratio is expected if males are subject to greater predation risk.

Gonopodium length exhibited moderate levels of phenotypic variation ($CV = 10.8\%$) compared to the average sexually selected display traits (see Pomiankowski and Møller 1995). In comparison, the phenotypic coefficient of variation for genitalic measures was 5.9% in the waterstrider *Gerris incognitus*, 8.1% in guppies (Kelly et al. 2000) and, on average, 6.5% in the 61 species of insect and spider examined by Eberhard et al. (1998). It is notable, however, that in *B. episcopi* eye and caudal peduncle width showed very similar levels of phenotypic variation to gonopodium length, although variation in eye width was actually far higher once the effects of allometry were controlled for.

As with genitalic traits in many insects (Eberhard et al. 1998), *B. episcopi* gonopodia showed negative allometric scaling, but so did eye width. The average slope for gonopodium length was intermediate between that for eye and caudal peduncle width. In contrast, guppy gonopodia showed positive allometry (Kelly et al. 2000), as do most non-genitalic sexual traits (cf. Petrie 1988, 1992, Green 1992). Eberhard et al. (1998) suggested that selection on a trait that stimulates a female tactilely favours intermediate, standard sized male genitalia, yielding low allometric values. This explanation is not applicable to *B. episcopi* because copulatory thrusting is fleeting and unlikely to involve "internal courtship". It is possible that a relatively long gonopodium is less efficient at sperm transfer but there are presently no data on this relationship, even for the extensively studied guppy. It is unlikely that a relatively longer gonopodium imposes substantially greater energetic costs on males (Brönmark and Miner 1992).

Sexual selection as an explanation

Poeciliid males have two main mating tactics (Farr 1989). They either court a receptive female prior to attempting copulation or force insemination through gonopodial thrusting. A few species only use courtship (4%), the majority only employ gonopodial thrusting (58%) and the rest use both tactics (38%) (Bisazza 1993). Gonopodia are relatively shorter in species with courtship than in those where thrusting is the only mating tactic (Rosen and Tucker 1961). The comparative data thus suggest that coercive mating selects for elongated gonopodia. Reynolds et al. (1993) also found that male guppies with relatively longer gonopodia more often attempted to engage in gonopodial thrusting. Coercive mating via gonopodial thrusting is the major, perhaps only, male mating tactic employed by free-living *B. episcopi* (Bisazza 1993).

Thus, one explanation for among-site variation in gonopodium length in *B. episcopi* is that increased length elevates male insemination success and selection is stronger in populations with greater predation risk. This explanation was proposed for the guppy where relative gonopodium length is greater in populations with higher predation risk (Kelly et al. 2000). In guppies, the argument is especially convincing because males use both mating tactics, but the relative frequency of gonopodial thrusting increases in high predation risk populations (Endler 1987, Magurran and Seghers 1994b). This must increase selection to achieve insemination via thrusting. Among-site variations in the mating tactics of *B. episcopi* are unknown, although life-history differences between sites with and without large predatory fishes (Jennions and Telford 2002) resemble those of the guppy (e.g. Reznick 1989). Even so, it is probable that environmental and ecological conditions affect sexual behaviour in poeciliids in similar ways (Bisazza 1993, Magurran et al. 1995, Magurran 1998, Leips and Travis 1999).

The sexual selection explanation has two weaknesses in explaining the pattern in *B. episcopi*. First, there was no correlation between the mean adult sex ratio and gonopodial length. We predicted a more male-biased sex ratio would lead to longer gonopodia. Although site-specific sex ratios were uncorrelated between wet and dry season samples, the mean sex ratio was actually more female-biased at *Characin* sites, where average gonopodium length is greater. Second, the difference between predator community types in average gonopodium length is small ($< 4\%$) and no study has shown that relative gonopodium length correlates with insemination success in poeciliids. In insects though, small differences ($< 10\%$) in the size of certain genital traits affect male mating (Preziosi and Fairbairn 1996) and fertilisation success (Arnqvist and Danielsson 1999).

Natural selection as an explanation

The correlation between gonopodium length and predators could also be due to environmental or biotic factors. First, selection for larger relative fin area in swimming fins to evade predators (sensu Godin 1997) may lead to a larger gonopodium as a pleiotropic effect. Second, as is common in stream environments (e.g. Endler 1995), downstream sites differ in various parameters, including the quantity and quality of mi-

crofauna, compared to upstream sites (Angermeier and Karr 1983). *Rivulus* sites tend to be upstream sites, where streams are smaller and flow rate is lower. Arnqvist and Thornhill (1998) have shown that the genital length is affected by food quality and quantity in a waterstrider (*G. incognitus*); and Batty and Lim (1999) showed that the gonopodium length of mosquitofish (*Gambusia affinis holbrooki*) is reduced by sewage effluent. Finally, Nicoletto (1996) has shown that phenotypic plasticity leads to stockier guppies with thicker caudal peduncles in water with faster flow rates.

One line of evidence consistent with an environmental explanation is that both sites on R. Frijolito were *Characin* sites, but males from the downstream site still had significantly longer gonopodia than those from the upstream site. We could not measure the length of other fins with accuracy (due to wear and tear and the lack of unambiguous basal termination), but we did find a small (1%) but significant increase in caudal peduncle width (which is essentially a measure of basal tail fin width) at *Characin* sites. This increase in tail width is, however, not indicative of greater overall stockiness in *B. episcopi* from *Characin* sites because there is no difference in body length-corrected mass between males at *Rivulus* and *Characin* sites.

Conclusion

In conclusion, gonopodium length (corrected for male body length) varies geographically in *B. episcopi* and is associated with predator community type. It is unknown if these differences are genetically based. It is, however, worth noting that genetic differences are not required for an adaptive scenario because population variation could be due to adaptive phenotypic plasticity. Our results resemble those of Kelly et al. (2000) for the guppy. Male guppies are under strong sexual selection whose form varies predictably with local predator community type (reviewed by Houde 1997). Kelly et al. (2000) therefore offered a sexually selected explanation for population variation in genital size in guppies. The same explanation may also account for the pattern in *B. episcopi*. However, we also provide an alternative explanation (selection on body shape or fins) that does not invoke sexual selection on genitalia. The relevance of this explanation should be directly tested in guppies. Do guppies from high-predation risk sites generally have larger fins? Or are they heavier for a given body length, so that body length underestimates total biomass? Even if the answers to these questions are in the negative, it remains to be shown that gonopodium length determines fertilisation success in poeciliids. Although the underlying causes of the patterns we have reported remain elusive, as more data are published on population variation in genitalic traits and general trends emerge, explanatory relationships should become more readily apparent.

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